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1 **Nanoscale synapse organization and dysfunction in**
2 **neurodevelopmental disorders**

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12 **keywords**

13 Receptor trafficking, synapse nanoscale organization, synaptic plasticity, neurodevelopmental
14 diseases, intellectual disabilities, autism spectrum disorder

15 **Abstract**

16 Neurodevelopmental disorders such as those linked to intellectual disabilities or autism spectrum
17 disorder are thought to originate in part from genetic defects in synaptic proteins. Single gene
18 mutations linked to synapse dysfunction can broadly be separated in three categories: disorders of
19 transcriptional dysregulation, disorders of synaptic signaling and disorders of synaptic scaffolding and
20 structures. The recent developments in super-resolution imaging technologies and their application to
21 synapses have unraveled a complex nanoscale organization of synaptic components. On the one hand,
22 part of receptors, adhesion proteins, ion channels, scaffold elements and the pre-synaptic release
23 machinery are partitioned in subsynaptic nanodomains, and the respective organization of these
24 nanodomains has tremendous impact on synaptic function. For example, pre-synaptic
25 neurotransmitter release sites are partly aligned with nanometer precision to postsynaptic receptor
26 clusters. On the other hand, a large fraction of synaptic components is extremely dynamic and
27 constantly exchanges between synaptic domains and extrasynaptic or intracellular compartments. It
28 is largely the combination of the exquisitely precise nanoscale synaptic organization of synaptic
29 components and their high dynamic that allows the rapid and profound regulation of synaptic function
30 during synaptic plasticity processes that underlie adaptability of brain function, learning and memory.
31 It is very tempting to speculate that genetic defects that lead to neurodevelopmental disorders and
32 target synaptic scaffolds and structures mediate their deleterious impact on brain function through
33 perturbing synapse nanoscale dynamic organization. We discuss here how applying super-resolution
34 imaging methods in models of neurodevelopmental disorders could help in addressing this question.

35

36 Introduction

37

38 Our brain consists of billions of neurons connected in a finely tuned network to ensure integration of
39 environmental stimuli, their processing and transformation into coordinated motor actions. Following
40 the initial proposition by Ramon y Cajal (Ramon y Cajal, 1899), the sites of communication between
41 single neurons – the synapses – have emerged as key players in information processing and storage by
42 the brain through a whole variety of synaptic plasticity processes. In the developing brain, new synaptic
43 connections are formed, strengthened or pruned depending on the inputs they receive over time. In
44 the developing and the mature brain, synapses continuously undergo activity dependent potentiation
45 and depression of their efficacy and these events are believed to play a central role in memory
46 formation and forgetting. All these processes result in a balanced interplay between neuronal
47 excitation and inhibition that allows adequate information processing by the normal brain.

48 Synapse function is ensured by a machinery of densely packed proteins in the pre- and the post-
49 synapse organizing neurotransmitter release in the presynapse and receptors on the postsynaptic site.
50 A key feature of synaptic function, that was recognized early on, is that the efficacy of synaptic
51 transmission is dictated by a combination of factors including primarily the amount of transmitter
52 released and the number of receptors present in front of these transmitter release sites. A recent study
53 further re-enforced this notion by elegantly showing the tight link between synapse structure and
54 function (Holler et al., 2021). Consequently, plasticity of synaptic transmission has long been thought
55 to involve mostly changes in transmitter release efficacy and/or receptor numbers at synapses (Nicoll,
56 2017).

57 Recent work by our lab and others (reviewed in (Biederer et al., 2017; Groc and Choquet, 2020)), using
58 largely super-resolution imaging, has uncovered two important features of neurotransmitter receptors
59 that complexify this initial view. First, receptors are not all static in the postsynaptic membrane, but a
60 large fraction alternate constantly between thermally powered random diffusion in the plane of the
61 membrane and immobile states stabilized through binding to scaffold proteins (Triller and Choquet,
62 2005). Second, many receptor subclasses are not randomly distributed in the postsynaptic density, but
63 rather are organized at the nanoscale in nanodomains (Crosby et al., 2019; Fukata et al., 2013;
64 Goncalves et al., 2020; Kellermayer et al., 2018; MacGillavry et al., 2013; Nair et al., 2013; Specht et
65 al., 2013). Furthermore, these receptor nanodomains seem to be organized with respect to transmitter
66 release sites (Haas et al., 2018; Hruska et al., 2018; Tang et al., 2016). This may prove to be particularly
67 important for low affinity receptors such as the AMPA subtype of glutamate receptors that mediate
68 most fast excitatory neurotransmission. Indeed, for these receptors, a change in localization of a few
69 tens of nanometer with respect to transmitter release sites has a strong impact on their probability to
70 be activated (Haas et al., 2018). Hence, altogether, the number of receptors activated during
71 transmitter release that sets synapse efficacy results from a dynamic equilibrium between receptors
72 present in synaptic, extrasynaptic and intracellular compartments and from their precise nanoscale
73 localization in the post-synapse (Groc and Choquet, 2020).

74 Regulation of receptors surface expression, recycling, trafficking and diffusion during synaptic plasticity
75 involves a large fraction of the interactome of glutamatergic synapses (Schwenk et al., 2012; Xu et al.,
76 2021). Among this interactome, scaffold proteins that bind receptor intracellular domains play a
77 central role by providing slots for receptor stabilization and accumulation at synapses in front of
78 transmitter release sites (Chen et al., 2018; Choquet and Hosy, 2020). A major process to regulate
79 receptor binding to their scaffolds is signaling-mediated post-translational modifications of receptors
80 and/or scaffolds that change receptor-scaffold affinities (e.g. for the major glutamatergic synapse
81 scaffold protein PSD-95 (Fukata et al., 2013; Hafner et al., 2015)). Along these lines, a fascinating new

82 emerging concept derives from the observation that receptors and scaffolds can partition in liquid
83 phases, thus creating biochemical grounds for the formation of nanoscale domains in the synaptic
84 density that can have important functional roles (Zeng et al., 2019; Zeng et al., 2016). Indeed, scaffold
85 proteins regulate not only clustering of receptors but also connect signaling pathways to downstream
86 targets which regulate translation (Good et al., 2011). In this framework, scaffold proteins play a
87 central role in the integration of synaptic inputs into downstream signaling in dendrites all the way to
88 the nucleus and this enables adaptation of protein expression, up- or downscaling of receptor density
89 and organization at the surface of the postsynaptic site (Iasevoli et al., 2013).

90 Noteworthy, the synapse interactome is composed not only of receptor subunits and scaffold proteins,
91 but also includes adhesion proteins, ion channels and catalytically active enzymes like kinases, GEFs
92 and GAPs. However, one striking characteristic of the postsynaptic density (PSD) is the core of various
93 densely packed scaffold proteins *e. g.* MAGUKs, DLGAPs and SHANKs facilitating and regulating
94 protein-protein interactions (Li et al., 2017) that seem to act as both organizational and signaling hubs.

95 Synapse dysfunction has been hypothesized to be involved in many brain diseases (Volk et al., 2015),
96 from neurodegenerative diseases such as Alzheimer's disease that was proposed to be a "synaptic
97 failure" (Selkoe, 2002) or some forms of schizophrenia with an autoimmune basis (Jezequel et al.,
98 2018) to intellectual disability (ID), childhood epilepsy, developmental delay (DD) or autistic spectrum
99 disorders (ASD), particularly since the first identification of an ASD related mutation in a synaptic
100 adhesion protein (Jamain et al., 2003). However, it is in most cases particularly difficult to decipher
101 whether synapse dysfunction is a cause or consequence of a given disease.

102 Alterations in the genome due to *de novo* or inherited mutations that cause dysfunction in synaptic
103 proteins is emerging as a major cause of neurodevelopmental diseases and provide the opportunity to
104 directly link synapse dysfunction to brain disease. A point mutation causing a stop codon can lead to
105 an early truncation of the protein which can result in expression of a protein with altered function or
106 "loss-of-function" due to nonsense mediated decay. Truncation can lead to a "gain-of-function" as
107 localization or regulation of this protein can be disturbed (Kervestin and Jacobson, 2012; Miller and
108 Pearce, 2014). Either way, the highly organized balance of the synaptic interactome is disturbed which
109 can have drastic consequences for the developing brain (Na et al., 2013).

110 Single gene mutations linked to synapse dysfunction can broadly be separated in three categories:
111 disorders of transcriptional dysregulation, disorders of synaptic signaling and disorders of synaptic
112 scaffolding and structures. Regarding the latter, in the context of our new emerging understanding of
113 the nanoscale organization of synapses, it is tempting to speculate that synapse dysfunction could
114 originate from changes not only in expression levels of given constituents but also on their nanoscale
115 organization. Alteration in some key synaptic proteins could lead to subtle re-organizations in synaptic
116 protein distribution that could profoundly impact synapse function but may only be revealed by
117 detailed analysis at the subsynaptic level by high resolution imaging. Here, we will review examples of
118 extensively studied postsynaptic proteins that are targeted in neurodevelopmental disorders and are
119 potential candidates for an impact on excitatory synapse nanoscale organization.

120 **A brief summary of synapse nanoscale dynamic** 121 **organization, function and dysfunction**

122 Synapse nanoscale organization has initially been studied using post-embedding immuno-gold
123 labelling and electron microscopy that provided the first view of receptors subsynaptic organization
124 (Baude et al., 1995; Baude et al., 1993; Lujan et al., 1996; Nusser et al., 1994; Triller et al., 1985).
125 Despite its power, this approach however bears severe limitations including a limited sensitivity and a
126 restriction to fixed tissue imaging. Sensitivity was greatly improved by the development of freeze

127 fracture replica labelling that can detect above 90 % epitopes and revealed a subsynaptic organization
128 of glutamate receptors (Masugi-Tokita and Shigemoto, 2007). In parallel, application of single particle
129 and molecule tracking techniques to follow receptor movements with nanometer resolution in live
130 neurons revealed that neurotransmitter receptors are not immobile in the synapse as was previously
131 thought but for a large fraction constantly exchange between mobile and immobile states (Borgdorff
132 and Choquet, 2002; Meier et al., 2001). While these approaches lacked the labelling density necessary
133 to obtain an adequate view of synapse nano-organization, they already revealed that receptors can be
134 confined in nanodomains, suggesting a subsynaptic organization. The advent of super-resolution
135 optical microscopy techniques, which are able to overcome the optical diffraction limit, opened the
136 way to an easier analysis of synapse nanoscale organization, and furthermore compatible with live
137 imaging. In particular, single molecule based super-resolution microscopy such as STochastic Optical
138 Reconstruction Microscopy (STORM) (Rust et al., 2006) and direct STORM (dSTORM) (Heilemann et al.,
139 2008), Photo Activation Localization Microscopy (PALM) (Betzig et al., 2006; Hess et al., 2006) or
140 universal Point Accumulation in Nano Topography (uPAINT) (Giannone et al., 2010) allow to detect
141 single molecule localization with a precision down to 10-20 nm, allowing to reconstruct synapse
142 organization at that precision. These further allow to count single molecules and obtain a precise
143 quantitative view of the number of molecules present in synaptic and sub-synaptic compartments.
144 Other deterministic super-resolution approaches that have been extensively used to investigate
145 synapse nano-organization include Structured Illumination Microscopy (SIM) (Gustafsson, 2000; Wu
146 and Shroff, 2018) and STimulated Emission Depletion (STED) (Hell and Wichmann, 1994; Vicidomini et
147 al., 2018) microscopy.

148 Using a variety of single molecule tracking and super-resolution approaches alone or in combination,
149 a wealth of studies has reported the nanoscale dynamic organization of synaptic components and how
150 this could contribute to synaptic function (Figure 1). These results have been reviewed extensively
151 (Biederer et al., 2017; Chamma et al., 2020; Groc and Choquet, 2020; MacGillavry and Hoogenraad,
152 2018; Maynard and Triller, 2019; Padmanabhan et al., 2020; Petit-Pedrol and Groc, 2021; Petrini and
153 Barberis, 2014). In summary, single particle or single molecule tracking studies applied to AMPA
154 (Borgdorff and Choquet, 2002; Tardin et al., 2003), NMDA (Groc et al., 2007) glutamate receptors or
155 metabotropic glutamate receptors (mGluRs) (Ferraguti and Shigemoto, 2006; Huang and Thathiah,
156 2015; Siddig et al., 2020), to glycine (Meier et al., 2001) or GABA (Bannai et al., 2009; Jacob et al., 2005)
157 inhibitory receptors, or to adhesion proteins (Chamma et al., 2016; Moretto et al., 2019) have first
158 established that all these synaptic proteins are in a dynamic equilibrium and constantly exchange
159 between the synaptic and extrasynaptic regions, alternating between mobile and immobile states.
160 Similarly, pre-synaptic components are also highly mobile and their dynamic contributes to control
161 pre-synapse function. For example, surface mobility of voltage-dependent calcium channels modulates
162 release probabilities of synaptic vesicles by supporting calcium domain cooperativity (Schneider et al.,
163 2015). Syntaxin1A is another critical element of the transmitter release machinery. It is organized in
164 nanoclusters that are critical for the docking and priming of secretory vesicles from neurosecretory
165 cells (Sajman et al., 2017; Sieber et al., 2006) and its lateral diffusion reveals transient and local SNARE
166 interactions that dynamically regulate neurotransmitter release (Bademosi et al., 2017; Ribault et al.,
167 2011). Munc18-1 and syntaxin-1A interact to act as diffusional switches that controls SNARE complex
168 assembly during priming of vesicles (Kasula et al., 2016).

169 Proteins are stabilized through interactions with intracellular scaffold elements, extracellular matrix
170 proteins or adhesion proteins. While all transmembrane proteins exhibit some proportion of freely
171 moving behavior, the ratio of mobile to immobile protein is highly variable. For example, for glutamate
172 receptors, NMDA type receptors containing the NR2A subunit exhibit the highest proportion of
173 immobile receptor, while mGluR1 or mGluR5 exhibit the highest mobility. NR2B containing receptors
174 and AMPA type receptors display on average 30-40% immobile receptors (reviewed in (Choquet and

175 Triller, 2013)). Most interestingly, for all transmembrane proteins studied, the fraction of mobile
176 receptors is highly regulated by a variety of processes, including neuronal development and neuronal
177 activity. For example, AMPA type receptors surface diffusion is negatively regulated by activity
178 dependent increases in intracellular calcium (Borgdorff and Choquet, 2002). This effect is mediated by
179 calcium induced activation of CaMKII which in turn phosphorylates the AMPAR associated auxiliary
180 subunits TARPs and increases their affinity for the scaffold protein PSD-95, leading to their
181 immobilization at synapses (Hafner et al., 2015; Opazo et al., 2010). This activity dependent trapping
182 of surface diffusing AMPAR at synapses is thought to play a major role in activity dependent
183 potentiation of synaptic transmission during LTP.

184 More recently, the advent of super-resolution imaging methods has led to a still developing extensive
185 characterization of synapse nanoscale organization. For example, AMPA receptors were shown to be
186 in part organized in nanodomains containing ~30 receptors (Goncalves et al., 2020; Nair et al., 2013).
187 A similar organization was observed for inhibitory receptors. Most interestingly, these nanodomains
188 were found to be localized in front of neurotransmitter release sites (Tang et al., 2016), likely through
189 transsynaptic adhesion proteins (Haas et al., 2018). This organization is regulated by synaptic plasticity,
190 synaptic depression being associated to a disorganization of these nanocolumns, while synaptic
191 potentiation is associated to an increase in the number of synaptic nanomodules (Hruska et al., 2018).
192 NMDA receptors also display fascinating nanoscale synaptic organization: GluN2A- and GluN2B-
193 NMDARs are organized in distinct nanodomains, which are differentially regulated by interaction with
194 PDZ scaffolds. This GluN2A/2B nanoscale organization bi-directionally tunes synaptic LTP (Kellermayer
195 et al., 2018). The increase in GluN2B-NMDAR surface dynamics after stimuli that induce LTP directly
196 contributes to the accumulation of α -CaMKII within the spine head (Dupuis et al., 2014). The highly
197 diffusive GluN2B-NMDAR could “shuttle” α -CaMKII to the PSD through their direct binding, consistent
198 with the well-defined and key role of the interaction between NMDAR and α -CaMKII into LTP and
199 learning processes (Bayer and Schulman, 2019).

200 Similar findings were obtained at inhibitory GABAergic synapses showing that neuronal activity
201 modifies the diffusion properties of type-A GABA receptors (Bannai et al., 2009; Petrini et al., 2014). In
202 this conceptual framework, a study focusing on kainate receptors showed that CaMKII-dependent
203 phosphorylation of GluK5 mediates plasticity of kainate receptors by markedly increasing their lateral
204 mobility, possibly by decreasing the binding of GluK5 to PSD-95 (Carta et al., 2013).

205 Modulation of synaptic transmission also involves metabotropic Glutamate receptors (mGluRs) which
206 are highly abundant in the brain, expressed pre- and postsynaptically and regulate long-term signaling
207 pathways (Huang and Thathiah, 2015). Group I mGluRs (mGluR1/5) are found at postsynaptic sites,
208 group II mGluRs (mGluR2/3) are present pre- and postsynaptically, and group III mGluRs (mGluR4,
209 mGluR6-8) are localized mainly at presynaptic sites (Ferraguti and Shigemoto, 2006; Shigemoto et al.,
210 1996). mGluR4 is highly expressed in the synapses of parallel fibers in the cerebellum which form
211 synapses with Purkinje cells and negatively regulate glutamate at the presynapse (Corti et al., 2002).
212 This receptor group is associated with neuronal survival, and data obtained from mice demonstrated
213 its significant role in synaptic plasticity and motor performance (Borodezt and D'Mello, 1998;
214 Pekhletski et al., 1996). To characterize the nano-organization of mGluR4 at parallel fiber active zones
215 (AZs), a study used dSTORM to define mGluR4 distribution with a precision of approximately 13 nm.
216 This study confirms previous reports based on freeze-fracture replica EM that mGluR4 is highly
217 organized in nanoclusters inside AZs where it is also 4 times more concentrated compared to outside
218 (Indriati et al., 2013). The authors were further able to estimate the abundance of monomers, dimers
219 and higher order nanoclusters, and showed that there are ~35 mGluR4 subunits per AZ organized in
220 ~25 nanodomains (Siddig et al., 2020). Performing nearest neighbor analysis they also explored the co-
221 organization of mGluR4 with the presynaptic Bassoon, Munc-18-1 and Cav2.1 channels (Siddig et al.,

222 2020). This study demonstrates how previous data obtained via conventional microscopy, but also EM
223 can be supported and strengthened by powerful methods like dSTORM. The mGluR glutamate receptor
224 subtype is very versatile and involved in various functions ranging from modification of transmitter
225 release via regulation of trafficking of ionotropic GluRs to induction of LTP and LTD (Ferraguti and
226 Shigemoto, 2006). Detailed knowledge about the diffusion properties and nanoscale organization of
227 mGluRs will help us to shed light on their role in modulation of synaptic function and activity-
228 dependent long-term scaling as well as their implication in disease phenotypes.

229 The precise, activity-dependent nanoscale co-organization of the three main glutamate receptor
230 subtypes AMPARs, NMDARs and mGluRs is the basis for modulation of synaptic transmission. Using
231 dual-color dSTORM we were able to show that, in the PSD, AMPARs are co-organized with NMDARs
232 but barely co-localize; interestingly, NMDAR clusters were rather localized to the center of the PSD and
233 surrounded by AMPAR clusters (Goncalves et al., 2020). Measuring the distribution of AMPARs and
234 mGluR5 revealed that mGluR5 is not organized in clusters. On the contrary, there were more mGluR5
235 objects detected at the perisynapse, outside AMPAR enriched areas and in dendrites which is in line
236 with other studies and might resemble their high lateral diffusion (Lujan et al., 1996) (Goncalves et al.,
237 2020). This report of fine-tuned co-organization between AMPA and NMDARs was confirmed by
238 studies using electron microscopy (Li et al., 2021). This group further observed that synchronous
239 release sites are aligned with AMPAR clusters and asynchronous release sites with NMDAR clusters
240 facilitating activation of NMDARs due to a AMPA-mediated depolarization of the membrane (Li et al.,
241 2021). This finding indicates that the scaffold machinery can discriminate between various receptor
242 types and is able to organize them – also in accordance with the presynaptic release sites which plays
243 a significant role in their efficiency and during synaptic plasticity.

244 In parallel to the increase in our knowledge of normal synapse dynamic nanoscale organization, several
245 studies have started to interrogate the potential link between synapse dysfunction in disease models
246 and alterations in its nano-organization and dynamic of its components. A first study reported altered
247 AMPAR trafficking by the stress hormone corticosterone that could explain the deficit in synaptic
248 plasticity during stress (Groc et al., 2008). More recently, we reported increased AMPAR surface
249 diffusion in models of Alzheimer’s (Opazo et al., 2018) and Huntington’s diseases (Zhang et al., 2018).
250 In both cases, the altered activity dependent AMPAR diffusion trapping at synapses could underlie the
251 deficits in synaptic plasticity that could in turn be responsible for the learning deficits in these models.
252 These defects in AMPAR surface trafficking are however likely to be only secondary to these
253 neurodegenerative diseases rather than their *primum movens*.

254 Altered receptor trafficking may also be responsible for defects in synaptic plasticity in some
255 psychiatric diseases. The discovery of the presence of circulating autoantibodies against glutamate
256 NMDA receptor (NMDAR-Ab) in about 20% of psychotic patients diagnosed with schizophrenia led to
257 the finding that NMDAR-Ab from patients, but not from healthy subjects, specifically alters the surface
258 dynamics and nanoscale organization of synaptic NMDAR, and this most likely explains that NMDAR-
259 Ab prevent long-term potentiation at glutamatergic synapses (Jezequel et al., 2017). There also, the
260 alteration in receptor traffic and synaptic function are secondary to the production of auto-antibodies.

261 The situation may be different in the case of highly penetrant mutations that occur in genes that are
262 critical regulators of synaptic function, and thus represent the primary cause of the disease (Reviewed
263 in (Bhakar et al., 2012; Parenti et al., 2020)). The most common single-gene mutations in ASD with ID
264 are associated with fragile X syndrome (*Fmr1*), tuberous sclerosis (*TSC1*, *TSC2*), neurofibromatosis
265 (*NF1*), Angelman syndrome (*UBE3A*), Rett syndrome (*MECP2*), the PTEN hamartoma tumor syndrome,
266 and Phelan-McDermid syndrome (*SHANK3*) (for review, see (Betancur, 2011)). Rare Mutations in the
267 neuroligin (*NLGN3*, *NLGN2*) and neurexin (*NRXN1*) genes also cause autism (Jamain et al., 2003).

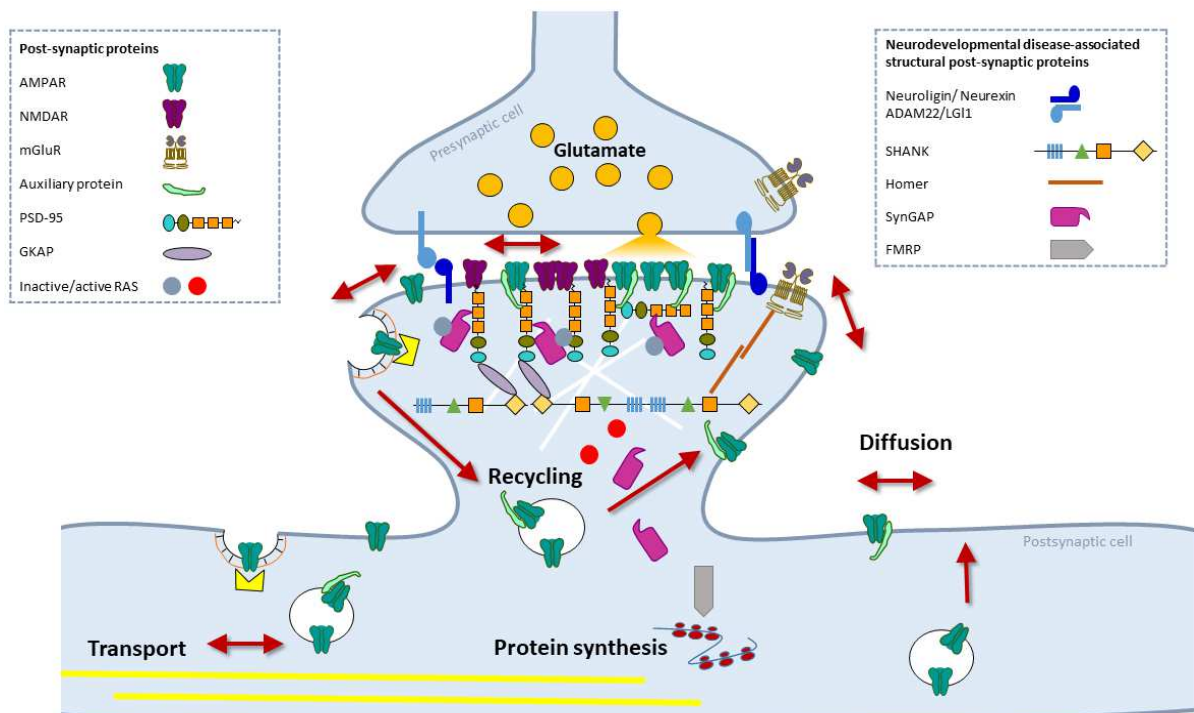
268 Several of these proteins are centrally involved in synapse organization and thus represent fascinating
269 models to link synapse nanoscale organization and function.

270

271 Disorders of synaptic scaffolding and structures

272 The core protein interaction machinery of the PSD includes members of the scaffold families DLG,
273 GLGAP and SHANK containing ANK, PH, PDZ and SH domains. These are crucial for protein-protein
274 interaction and localization (Li et al., 2017). Disruption of interactions or mis-localization of
275 components of this protein complex due to mutations can have drastic consequences for the whole
276 synaptic protein interaction network. It is thus not surprising that disruption of many of the synaptic
277 proteins is involved in brain disorders, particularly at early stages. Indeed, already at prenatal stages,
278 during synaptogenesis, the core scaffold structure is establishing and serves as a hub to cluster the
279 different molecular complexes of the developing PSD (Li et al., 2017). In the developing brain, synapses
280 are formed and synaptic transmission is adapted due to stimuli inducing LTP or LTD. Homeostatic
281 plasticity mechanisms ensure stable function of neuronal circuits during experience-dependent
282 development. More precisely, postsynaptic excitatory strength can be scaled up or down accordingly
283 to compensate for changes in neuronal firing (Turrigiano et al., 1998). This fine-tuned adaptation can
284 be disturbed by dysfunction of synaptic proteins and it can be difficult to differentiate between an
285 activity-induced up- or downscaling of receptors, a pathological phenotype or a compensatory
286 mechanism due to receptor levels outside the norm. In the following paragraphs we will illustrate by
287 some examples how dysfunction of disease-associated proteins can affect synaptic function, alters
288 transmission efficiency and homeostasis, and causes neurodevelopmental disorders. And finally
289 delineate, how powerful imaging techniques can help to better understand the impact dysfunction of
290 proteins can have on synapse maturation and nano-organization.

291



292

293 **Figure 1:** Overview of a glutamatergic synapse depicting the organization of postsynaptic proteins according to
294 our current knowledge, highlighting some of the prominent targets of neurodevelopmental genetic defects
295 involving structural synaptic proteins.

296 SHANK as a general synapse organizer and main target in ASD

297 The SH3 and multiple Ankyrin repeat domain family members (*Shank 1-3*) are multidomain scaffold
298 proteins that play a vital role in the PSD of glutamatergic synapses where they interact with many other
299 scaffolding and signaling proteins. Together with Homer, SHANK proteins build the network of scaffold
300 proteins that sits deeper in the PSD (Kim and Sheng, 2004). Via its interaction with GKAP and PSD-95,
301 SHANK anchors indirectly a complex connecting AMPARs, NMDARs and mGluRs and mediates
302 downstream signaling being in contact with the actin cytoskeleton (Naisbitt et al., 1999). The three
303 members of the SHANK family are expressed in different brain regions and are involved in distinct
304 functions.

305 Patients with alterations in Shank protein levels due to haploinsufficiency or duplication have
306 phenotypes like Phelan–McDermid syndrome (PMDS), global developmental and language delay, ASD
307 and ID with different degrees of severity (Bourgeron, 2015; Mossa et al., 2021; Zoghbi and Bear, 2012).
308 Numerous studies on *Shank1*-, *Shank2*- and *Shank3*-mutant mice demonstrated that loss of Shank
309 affects spine size and number, dendritic branching as well as glutamatergic signaling, AMPA-mediated
310 synaptic transmission and homeostatic plasticity (Monteiro and Feng, 2017; Tatavarty et al., 2020). A
311 study using induced pluripotent stem cells (iPSCs) from patients with *Shank2* haploinsufficiency
312 showed that low SHANK2 levels caused an overall neuronal hyperconnectivity due to a higher synapse
313 number as well as an increased branching complexity and dendrite length (Zaslavsky et al., 2019).
314 Another study on *Shank1-3* double knock out mice described an increased mortality around P22-P25,
315 which is a time window during development crucial for synapse formation and maturation. Treatment
316 of those mice with cotinine to increase Akt activity improved their survival rates, as well as sociability
317 and cognitive performance. Interestingly, cotinine was also successfully used in a mouse model for
318 Fragile X syndrome (Pardo et al., 2017).

319 Several studies exploring loss of *Shank3* in different models report alterations in synaptic properties
320 and propose various strategies of pharmacological intervention ameliorating the phenotypes.
321 Recently, one group reported that loss of *Shank3* disrupts synaptic scaling and intrinsic homeostatic
322 plasticity *in vitro*. They further demonstrated that neocortical neurons of *Shank3* knockout mice were
323 strongly impaired to encounter homeostatic recovery of the visual cortical circuit after perturbations
324 to sensory drive. Interestingly, the impact on basal synaptic properties was minor which emphasizes a
325 significant role of *Shank3* in homeostatic plasticity. Treatment with Lithium – a GSK3 target - rescued
326 the deficits observed *in vitro* and improved the robust self-grooming phenotype in *Shank3* knockout
327 mice implying that loss of *Shank3* mis-regulates the GSK3 pathway and thus disturbs homeostatic
328 compensation (Tatavarty et al., 2020).

329 Interestingly, another group generated iPSC-derived neurons lacking *Shank3* from patients with PMDS
330 and ASD and showed that those neurons present major defects in AMPA- and NMDA-mediated
331 synaptic transmissions, whereas inhibitory synaptic transmission was not impaired. They were able to
332 rescue the observed defects in those neurons by either restoring SHANK3 levels or by treatment with
333 insulin-like growth factor 1 (IGF1) which stimulates formation of excitatory synapses containing PSD-
334 95 and NMDARs (Shcheglovitov et al., 2013). Resembling this finding, another group generated human
335 neurons derived from embryonic stem (ES) cells and introduced *Shank3* deletions to compare isogenic
336 control with heterozygous and homozygous mutant neurons. They observed a decrease in both,
337 evoked excitatory postsynaptic currents (EPSCs) and in the amplitude of spontaneous miniature EPSCs
338 in the *Shank3*^{+/-} neurons which was similar but more severe in the *Shank3*^{-/-} neurons. Next to
339 alterations in synaptic properties, they also observed changes in intrinsic electrical properties resulting
340 in an increased input resistance in *Shank3*-mutant neurons. They proposed HCN channel-mediated I_h
341 currents as explanation as HCN channels reduce membrane resistance by depolarizing the membrane
342 toward the action potential threshold (Benarroch, 2013). Confirming this hypothesis, treatment with
343 an I_h -current inhibitor caused an increase in input resistance also in wildtype neurons (Yi et al., 2016).
344 Further experiments revealed that SHANK3 proteins interact with HCN channels and showed an

345 impairment of I_h currents in *Shank3*-mutant neurons compared to wildtype neurons, leading to the
346 conclusion that the impairment in intrinsic properties affects also synaptic function (Yi et al., 2016).
347 In the same year the role of *Shank3* in excitatory synapse transmission of dopamine (DA) and GABA
348 neurons was explored by downregulating SHANK3 levels post-natally using an shRNA approach
349 (Bariselli et al., 2016). The reduction of SHANK3 levels was induced specifically in the ventral tegmental
350 area (VTA), a brain structure which seems to be involved in social and repetitive behavior. This affected
351 maturation of excitatory synapses onto DA and GABA neurons, accompanied by reduced burst activity
352 of DA neurons and increased activity of GABA neurons. Treatment during a critical time window of
353 synapse maturation with a positive allosteric modulator (PAM) of mGluR1 restored synaptic properties
354 and ameliorated social deficits (Bariselli et al., 2016) supporting the importance of VTA DA neurons
355 during development.

356 Those reports are exemplary and go in line with the deficits in synaptic scaling mentioned above as a
357 possible consequence of impaired synaptic properties and reduced ability to adapt to changes in
358 neuronal circuits.

359 The numerous studies that have highlighted the important role of SHANK proteins in organizing not
360 only various glutamate receptors but also scaffolding proteins in the PSD are a strong incentive to
361 explore the nanoscale changes and modifications in protein-protein co-organization induced by the
362 various gain and loss of function in the diverse SHANK isoforms. The number of mutations described
363 and the different models studied, including patients' cells, are a fantastic opportunity to understand
364 the complexity of SHANK proteins and can be explored in further depth using super resolution
365 techniques.

366

367 SYNGAP1 – regulator of synaptic plasticity

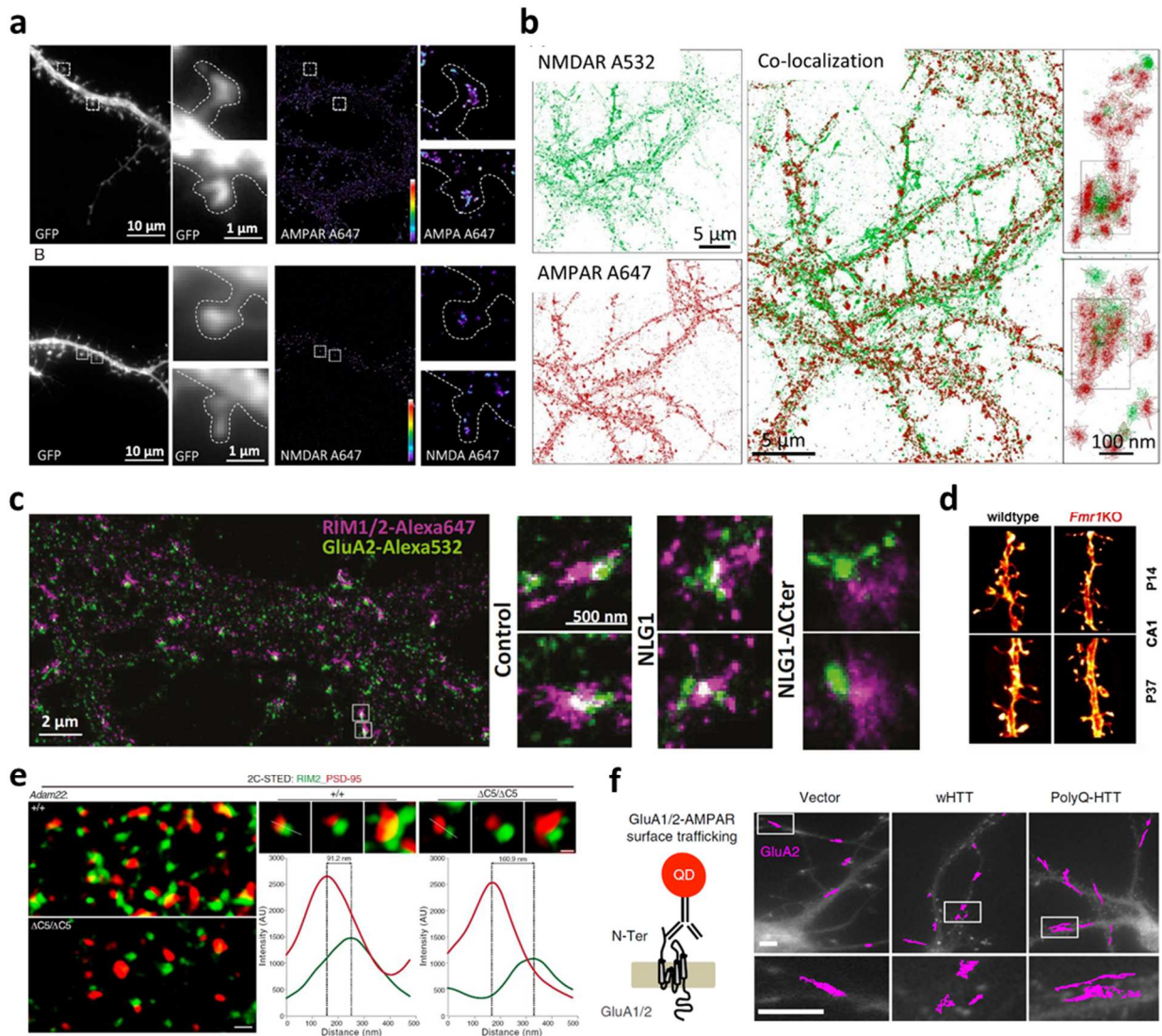
368 *SYNGAP1* is appearing as one of the most fascinating synaptic targets in ID. The synaptic GTPase
369 Activating Protein (SynGAP) is highly abundant in the brain and is specifically localized at excitatory
370 synapses. As its name indicates, SynGAP has enzymatic properties but it also acts as a scaffold protein.
371 SynGAP is expressed as several isoforms and splice variants with various N- and C-Termini which have
372 discrete functions and distinct spatiotemporal distribution (Gamache et al., 2020; Gou et al., 2020;
373 McMahon et al., 2012). One of these isoforms is the $\alpha 1$ isoform which interacts with all three PDZ
374 domains of PSD-95 and SAP102 via its C-terminal PDZ binding motif (Chen et al., 1998; Kim et al., 1998).
375 The GAP domain of SynGAP regulates the activity of the GTPases Ras and Rap which are via the Erk
376 pathway involved in endo- and exocytosis of AMPARs. Activation of CaMKII phosphorylates SynGAP
377 and induces its rapid dispersion out of the spine, resulting in a higher fraction of the GTPases Ras and
378 Rap present in their active form favoring insertion of AMPARs at the surface (Araki et al., 2015). Activity
379 dependent dispersion out of the spine also results in increased availability of the PDZ domains of PSD-
380 95 for other proteins like TARPs, NMDA-type glutamate receptors (NMDARs, neuroligins and LRRTMs
381 (Leucine Rich Repeat Trans Membrane proteins). Those competitive properties of SynGAP could
382 contribute to the composition of the PSD and receptor organization at the surface (Opazo et al., 2012
383 ; Walkup et al., 2016). Immature neurons lacking *SYNGAP1* show larger clusters of postsynaptic
384 proteins like PSD-95, GluA1 and NR1 compared to wildtype neurons. Also, upon SynGAP loss of
385 expression, the fraction of postsynaptic proteins localized in protrusions is higher (Vazquez et al.,
386 2004). Already at basal state the AMPAR content is at its upper limit which is represented by an
387 enhancement in AMPA-mediated synaptic transmission, drastically impairing the ability to insert more
388 AMPARs upon stimulation (Araki et al., 2015; Rumbaugh et al., 2006). These conditions favoring LTP
389 have a strong effect on synaptic plasticity during critical phases in neurodevelopment. Several studies
390 in rodents showed that loss of *SYNGAP1* causes an early maturation phenotype with increased in spine
391 -size and -density and hyperexcitability early in development (Clement et al., 2012; Gamache et al.,
392 2020; Vazquez et al., 2004). In humans, many of the *de novo* mutations are nonsense or frameshift
393 mutations causing nonsense mediated decay or a truncated protein that is dysfunctional (Gamache et

394 al., 2020). *SYNGAP1* haploinsufficiency is associated with ASD, developmental delay and intellectual
 395 disability and accounts for up to 1% of all nonsyndromic ID cases (Hamdan et al., 2009). Exploring the
 396 nano- and co-organization of GluRs at the synapse would help to interpret results obtained from
 397 comprehensive electrophysiological studies and to better understand how alterations of receptor
 398 organization influence synaptic properties and affect the neuronal network.

399

400

401



402

403 **Figure 2:** Examples of super-resolution images exploring the nanoscale organization of the synapse
 404 using models for health and disease (a) Nano-organisation of AMPA and NMDA receptors in primary
 405 neurons using dSTORM and (b) their co-organization using dual color dSTORM (Goncalves et al.,
 406 2020). (c) Dual color dSTORM images showing the impact of NLG1 modification on RIM1/2 (magenta)
 407 and GluA2 (green) co-organization (Haas et al., 2018). (d) STED microscopy images comparing the
 408 morphology of wildtype and *Fmr1KO* neurons at different developmental stages (Wijetunge et al.,
 409 2014). (e) Dual color STED images of neurons labeled for RIM2 (green) and PSD-95 (red) depicting the
 410 impact of the disease-associated protein ADAM22 on pre-postsynaptic alignment. Scale bars: 500
 411 nm, 200 nm, magnified. (Fukata et al., 2021). (f) Nanoparticle tracking approach using quantum dot
 412 (QD) to track endogenous GluA2- GluA1-AMPA surface diffusion (scheme). GluA2-QD trajectories

413 (magenta) in different Huntington disease models using hippocampal neurons. Zoom-in below. Scale
414 bars, 10 μm . (Zhang et al., 2018).
415

416 **Adhesion proteins organize transsynaptic alignment**

417 The interplay between the pre-and post-synapse is crucial for proper synaptic transmission. Adhesion
418 proteins were shown to tightly organize trans-synaptic alignment and are responsible for efficient
419 activation of GluRs by neurotransmitters (Biederer et al., 2017; Sudhof, 2018). Here we will give two
420 examples demonstrating how nano-organization can be disturbed by loss of only few amino acids and
421 how those changes can be explored using super-resolution microscopy.

422 **Neurologin**

423 Neuroligins (NLG) are a family of transmembrane adhesion proteins implicated in ASD and ID
424 (Bourgeron, 2015). The different neuroligin isoforms are located on the autosome (*NLGN1*, *NLGN2*)
425 and X chromosome (*NLGN3*, *NLGN4*). They differ in terms of expression localization and play a key role
426 during development (Sudhof, 2018). During synaptogenesis Neuroligin1 (NLG1) is involved in
427 formation of new synapses and later contributes to the organization of the postsynaptic compartment
428 where it is regulated by CaMKII or PKA phosphorylation (Heine et al., 2008; Letellier et al., 2018).
429 Typically, in X-linked brain disorders, mainly male patients are affected, the mothers can be carriers of
430 the mutation without showing any or mild symptoms. Disruption of NLGN function causes ASD and ID,
431 hyperactivity and cognitive deficits (Jamain et al., 2003; Nakanishi et al., 2017; Nguyen et al., 2020).
432 Alterations in *NLGN3* are linked to ASD caused by loss of *NLGN3* as well as an extensively studied R451C
433 point mutation which impairs intracellular trafficking of NL3 (Jamain et al., 2003; Nguyen et al., 2020).
434 Investigation of both *NLGN3* alterations in mice revealed an imbalance between excitation and
435 inhibition in D1-dopamine receptor-expressing medium spiny neurons. Additionally, repetitive
436 behavior of motor routines was observed in those mice suggesting that ASD-associated *NLGN3*
437 mutations disrupt striatal circuits resulting in the phenotype observed (Rothwell et al., 2014). Using
438 dual-color d-STORM super-resolution microscopy, we reported in 2018, how loss of function of NLG1
439 impacts transsynaptic organization and impairs normal AMPAR activation (Haas et al., 2018). NLG1,
440 which is localized to excitatory synapses, has been proposed to be implicated in alignment of vesicle
441 release sites on the presynaptic site and AMPA receptor clusters on the postsynaptic site. Taking
442 advantage of dual color super-resolution optical imaging it was possible to unravel the spatial
443 correlation between vesicle release at presynaptic boutons and organization of AMPAR clusters on the
444 postsynaptic site (Tang et al., 2016). The formation of these nanocolumns across pre- and post-synapse
445 is regulated by synaptic activity. By expressing a dominant negative mutant of NLG1 lacking the C-
446 terminus that abolished NLG1 binding to PSD-95, we could impair the transsynaptic alignment of the
447 presynaptic glutamate release machinery with AMPAR clusters, without affecting GluA2 density, and
448 demonstrate how this profoundly impacts synaptic transmission (Haas et al., 2018). To confirm this
449 finding without disturbing NLG1 function the effect of divalent biomimetic ligands comprising the 15
450 C-terminal amino acids of NLG1 were tested. The ligand was designed to compete with endogenous
451 neuroligins for PDZ domains of postsynaptic scaffold protein. When comparing the ligand to a
452 nonsense control the effects observed were very similar to the ones observed for the NLG1 mutant
453 (Haas et al., 2018).

454 **LGI1-ADAM22-MAGUK**

455 Another example demonstrating how crucial is the alignment of the presynaptic neurotransmitter-
456 release machinery and postsynaptic receptors for proper synaptic function, lies in the LGI1-ADAM22-
457 MAGUK protein complex. LGI1, a neuronal secreted protein, and its receptor ADAM22, play a
458 significant role in transsynaptic nano alignment and are essential for precise transsynaptic

459 transmission (Fukata et al., 2017). In humans, dysfunctional LGI1 gene causes autosomal dominant
460 lateral temporal lobe epilepsy. ADAM22 is implicated in infantile epileptic encephalopathy with
461 intractable seizures and ID (Fukata et al., 2021; Fukata et al., 2017). Loss of the C-terminal PDZ ligand
462 of ADAM22 abolished binding to PSD-95 and presents an epileptic phenotype. Dual color STED imaging
463 revealed that PSD-95 was positioned in close proximity to LGI1 and ADAM22 (Fukata et al., 2021). The
464 LGI1–ADAM22–MAGUK protein complex was investigated in an *Adam22^{ΔC5/ΔC5}* mouse line lacking the
465 C-terminal PDZ ligand of ADAM22 to disrupt binding to PSD-95. These mice presented an epileptic
466 phenotype and die suddenly after 60 days. Dual color STED imaging indicated that LGI1 clusters were
467 no more aligned with PSD-95 nanodomains, and intensity of PSD-95 in nanodomains as well as their
468 size were significantly decreased (Fukata et al., 2021). This study further demonstrated that
469 modulation of AMPA-mediated synaptic transmission was disturbed in *Adam22^{ΔC5/ΔC5}* mice, as
470 overexpression of PSD-95 failed to potentiate AMPAR-mediated EPSCs. This is in accordance with
471 previous data showing that PSD-95 does not influence AMPAR-mediated transmission in *Lgi1^{-/-}*
472 neurons (Lovero et al., 2015). In this study, it was further observed that the average nearest neighbor
473 distance between presynaptic RIM2 and postsynaptic PSD-95 nanodomains was significantly larger in
474 *Adam22^{ΔC5/ΔC5}* mice compared to the wildtype. Disruption of the transsynaptic alignment mediated by
475 LGI1-ADAM22 had a severe impact on both alignment and size of PSD-95 nanodomains, causing
476 seizures and sudden death in mice. This further suggests that PSD-95 is no longer capable of organizing
477 AMPARs in transsynaptic nanocolumns with AZs, which is crucial for synaptic plasticity and scaling
478 mechanisms.

479 Regulation of protein synthesis by FMRP

480 One extensively studied disease-associated gene is fragile X mental retardation 1 (*Fmr1*). Its
481 corresponding protein FMRP is not directly a structural protein in the synapse but an RNA binding
482 protein that regulates protein synthesis at postsynaptic sites of dendrites where it is involved in
483 synapse development and maturation (Huebschman et al., 2020). As FMRP acts as a translational
484 repressor, its loss results in an increase in protein synthesis. In line with this, it was shown that protein
485 synthesis downstream from mGluR5 is highly upregulated (Krueger and Bear, 2011; Zoghbi and Bear,
486 2012).

487 Fragile X was first described in 1943 by Martin and Bell who observed a constriction of the X
488 chromosome causing intellectual disability, DD, and ASD among other phenotypes (Martin and Bell,
489 1943; Zoghbi and Bear, 2012). Studies on postmortem human tissue revealed morphological changes
490 of dendritic spines. About eight decades later, a study using stimulated emission-depletion (STED)
491 imaging, explored spine morphogenesis comparing hippocampal neurons of *Fmr1* ko mice with
492 wildtype mice at different developmental stages (Wijetunge et al., 2014). In this study, neuronal
493 morphology of mice at P14 and P37 was assessed and no major differences between *Fmr1* ko and
494 wildtype mice were found. Another group, also using STED imaging upon 2-photon glutamate
495 uncaging, also observed no changes in nanoscale morphology or spine density when comparing
496 neurons in the somatosensory cortex of *Fmr1^{-/-}* with WT mice. However, *Fmr1^{-/-}* mice showed
497 increased action potential generation. Serial electron micrographs revealed that the number of multi-
498 innervated spines (MIS) were increased in *Fmr1^{-/-}* mice resulting in a higher number in AMPARs and
499 NMDARs (Booker et al., 2019). To investigate the role of FMRP early in development one group
500 compared hippocampal CA1 neurons at two different developmental time windows in *Fmr1* ko (Banke
501 and Barria, 2020). They found an upregulation of GluA2 subunits, an increased AMPAR-NMDAR ratio
502 as well as impaired LTP in *Fmr1* ko animals at P6-P9. Those changes normalized at 2 weeks postnatal,
503 suggesting a compensation and adaptation of CA1 neurons to the altered environment. Those reports
504 were supplemented by another study focusing on changes in lateral diffusion of endogenous mGluR5
505 in *Fmr1* ko mouse neurons using single nanoparticle (quantum dot) tracking which found an increase

506 in diffusion dynamics in the synaptic area of *Fmr1* ko neurons. They could further relate this
507 observation to deficits in Homer and mGluR5 binding (Aloisi et al., 2017). This study emphasizes once
508 more the impact that imbalance in expression levels and altered protein function can have on synaptic
509 receptor dynamic organization and neuronal circuit formation especially early in development, as it
510 seems to be the case with loss of *Fmr1*.

511

512 **Compensatory mechanisms**

513 Most of the proteins described here are synaptic proteins. Still, it is important to keep in mind that
514 also dysfunction of proteins that are not directly associated with synapses can have a strong impact
515 on synaptic maturation and function. One example we mention is FMRP which is an RNA binding
516 protein and regulates translation. Further, catalytically active proteins like transcription factors,
517 kinases, GTPases that are not brain specific but have diverse cellular functions are not exclusively
518 associated with neurodevelopmental disorders (Baltussen et al., 2018; Hettige and Ernst, 2019; Kunde
519 et al., 2013; Stornetta and Zhu, 2011).

520 In some cases, it is not very obvious, if synaptic alterations are the result of a dysfunctional protein or
521 rather the consequence of compensatory mechanisms taking place to restore synaptic function.
522 Compensatory mechanisms can take place early in development and it can be difficult to distinguish if
523 altered network function is due to alterations of a synaptic protein or if the role of the protein is more
524 global and synaptic changes are an adjustment to those cellular changes. A recent study compares four
525 models of ASD that all share an elevated synaptic excitation to inhibition (E-I) ratio. The authors show
526 that this increased E-I ratio reflects homeostatic stabilization of synaptic drive in order to maintain
527 synaptic depolarization and spiking excitability (Antoine et al., 2019).

528 It remains topic of future studies to understand in further detail if the synaptic changes observed are
529 the consequence of compensatory mechanisms or caused directly by malfunctional proteins.

530

531 **Outlook and conclusion**

532 The work currently performed on understanding neurodevelopmental disorders including ASD and ID
533 is multidisciplinary and will help us not only to understand the underlying cause of dysfunction and
534 altered behavior of individuals but will also help broaden our knowledge about the normal function of
535 the brain. The studies mentioned above illustrate that genetic mutations can result in protein
536 dysfunction which impacts synaptic properties by disturbing the fine-tuned synaptic machinery.
537 Deficits in synaptic physiology in turn impair neuronal circuit properties and synaptic scaling
538 mechanisms and plasticity, as reviewed in detail here (Mullins et al., 2016). Those defects have a severe
539 impact on the developing brain and can result in altered behavior, impaired cognitive function and also
540 seizures. The number of identified mutations associated with neurodevelopmental disorders is
541 enormous and keeps increasing. It is important to understand if and how genetic variations might
542 result in the same pathophysiology as they might be involved in the same signaling pathway or be
543 connected in their function.

544 The rapidly growing list of genetically associated neurodevelopmental diseases as well as the massive
545 emergence of the multigenic nature of many psychiatric diseases urgently calls for the development
546 of new approaches to better characterize the disease targets, their mechanism of impact on synaptic
547 dysfunction, understand the role of the genetic background on the penetrance of the mutations,
548 altogether to help elaborate therapeutic strategies.

549 Cellular models

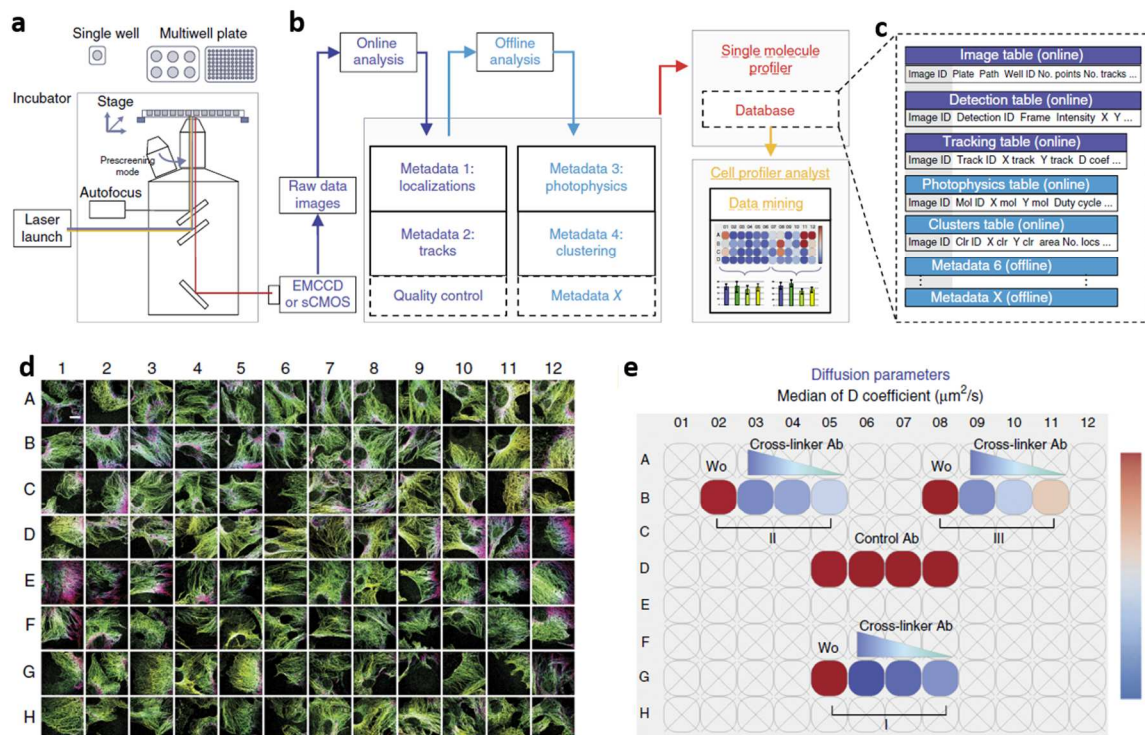
550 Patient derived induced pluripotent stem cells (iPSCs) are a quickly emerging model in neuroscience
551 (Aoi, 2016; Wen et al., 2014). Neurons derived from patients' cells resemble the situation in human
552 individuals and can bridge the gap between established rodent models and translational approaches
553 to explore deficits in synaptic nano-organization and morphology (Gouder et al., 2019). However, it is
554 important to take into account the genetic background which varies between individuals and makes it
555 even more challenging to compare the results observed, and to be sure to have only a single gene
556 mutation. One approach to overcome this variation is to use human iPSCs and introduce specific
557 mutations which resemble the situation in patients using CRISPR Cas9 or Cre/Lox technology- with the
558 advantage of having the identical genomic background (Li et al., 2016; Llamosas et al., 2020; Patzke
559 and Sudhof, 2016). As there are numerous neurodevelopmental disease models in rodents extensively
560 studied and characterized, those can serve as orientation and it is highly interesting to which extent
561 both models will be in accordance. Also, the development of organoids is an extremely powerful model
562 to recapitulate phenotypes that may not be observed in rodent models. Organoids resemble the highly
563 complex network of the brain better than two dimensional cultures do and will contribute to our
564 knowledge – especially combined with super-resolution imaging approaches (Chen et al., 2019;
565 Lancaster and Knoblich, 2014). One limitation might be that organoids do not represent the
566 connectivity and physiological properties found in a fully developed brain and might have limitations
567 in exploring neurodevelopmental disorders. Human and animal models have their strengths and
568 weaknesses and it is necessary to alternate between models to stay flexible in evaluating certain
569 phenotypes. Understanding the individual situation concerning alterations in nano-organization and
570 the underlying reasons opens new doors for personalized treatment which are still costly (Ferreira and
571 Mostajo-Radji, 2013; Stoddard-Bennett and Reijo Pera, 2019). However, high throughput studies using
572 hiPSCs are being implemented and might revolutionize medical treatment of neurodevelopmental
573 disorders. Development of reliable High Throughput Studies (HTS) will not only reduce costs for
574 personalized treatment but will also be a fast and robust approach by minimizing bias and
575 experimental variables.

576

577 High throughput imaging approaches

578 The large number of cellular models, gene mutations and experimental conditions that need to be
579 analyzed by super-resolution microscopy to investigate the impact on synapse nanoscale organization
580 calls for the use of high throughput imaging approaches. Super-resolution imaging approaches, and
581 particularly single-molecule localization microscopy techniques, have proven to be essential tools for
582 quantitatively monitoring biological processes at unprecedented spatial resolution. However, these
583 techniques are very low throughput and have only recently become compatible with fully automated,
584 multiparametric cellular assays. A High Throughput Study (HTS) characterized the activity-dependent
585 remodeling of the nanoscale architecture of the active zone and the postsynaptic density providing an
586 unbiased selection of 12,500 and 25,000 protein clusters. Using two-color STED images they
587 implemented Statistical Object Distance Analysis (SODA) in Python to quantify the coupling probability
588 of the postsynaptic scaffold proteins PSD-95 and Homer1c and the presynaptic scaffold proteins
589 Bassoon and RIM 1/2 (Wiesner et al., 2020). The coupling properties of Bassoon-PSD-95 and Bassoon-
590 Homer1c were comparable to the distances reported in previous studies with STORM (Dani et al., 2010;
591 Wiesner et al., 2020). To explore activity-dependent changes they identified the mean coupling
592 distances between all four pre- and postsynaptic pairs under application of external solutions either
593 driving NMDAR activation or reducing activity. They observed no difference in spatial organization of
594 the presynaptic pair but an increase in coupling distance between Homer1c and PSD-95. To assess the
595 impact of synaptic plasticity they induced LTD and LTP and found an increase in spatial organization
596 between PSD-95 and Bassoon upon LTP-induction and more randomly distributed cluster pairs after
597 application of an LTD stimulus. For a detailed analysis, they combined pySODA with unsupervised

598 machine learning approaches and extracted the following features: coupling probability and coupling
 599 distance, the area, eccentricity, minor axis length, major axis length, and perimeter using different
 600 stimulation protocols (Wiesner et al., 2020). We recently developed a HTS platform and pipeline for
 601 single-molecule localization approaches (Beghin et al., 2017) (Figure 3). It is an automated quantitative
 602 single-molecule-based super-resolution methodology that operates in standard multiwell plates and
 603 uses analysis based on high-content screening and datamining software. The workflow is compatible
 604 with fixed- and live-cell imaging and allows extraction of quantitative data like fluorophore
 605 photophysics, protein clustering or dynamic behavior of biomolecules. We have demonstrated that
 606 the method is compatible with high-content screening using 3D dSTORM and DNA-PAINT based super-
 607 resolution microscopy as well as single-particle tracking.
 608 Altogether, super-resolution HTS presents the framework to characterize activity-dependent changes
 609 in synaptic protein nanoscale organization of a large number of synapses. Combining this extremely
 610 promising approach with experiments determining the physiological characteristics of neuronal
 611 circuits will be crucial to understand the impact synaptic protein organization has in health and disease.
 612



613
 614 **Figure 3:** summary pipe-line for high content screening based on single molecule localization
 615 microscopy (HCS-SMLM) acquisition and analysis workflow. (a) Acquisition set-up. SMLM data are
 616 acquired on a conventional motorized inverted microscope. (b) Quantitative single molecule data are
 617 computed online during the acquisition (dark blue) and offline after the acquisition (cyan). The Single
 618 Molecule Profiler (SMP) software organizes all metadata into a unique database compatible with Cell
 619 Profiler Analyst (CPA) software. (c) HCS-SMLM metadata computed with SMP are composed of several
 620 tables linked together. (d) Fully automated 3D-dSTORM imaging of a complete 96-well plate. Each
 621 image is a 96 astigmatism-based 3D dSTORM images of a $20.5 \times 20.5 \mu\text{m}$ field of view, 40 nm per pixel
 622 of microtubules in Cos-7 cells automatically gathered using the HCS-SMLM approach. The total
 623 acquisition time was 8 h, including the localization and the reconstruction, and total traveling distance
 624 between the first and the last cell of the screen was 85 cm. The color codes indicate the Z-position. (e)
 625 Quantification of mobility and clustering of membrane proteins. Data mining of a HCS-SMLM
 626 experiment to measure the effect of antibody (Ab) mediated membrane protein cross-linking on their
 627 surface diffusion and showing heatmaps of median diffusion coefficient as a function of Ab cross-linker
 628 concentrations (Wo, without Ab cross-linkers). Reproduced from (Beghin et al., 2017).
 629

630 Antisense Oligonucleotides

631 Understanding the underlying reasons for a disorder is the first step towards its treatment. One
632 strategy we mentioned earlier is the restoration of protein levels. A technology that keeps maturing
633 and seems to be a very promising therapeutic application goes back to the late 70s when its application
634 was first published by Paul Zamecnik and Mary Stephenson (Agrawal, 2010; Stephenson and Zamecnik,
635 1978; Zamecnik and Stephenson, 1978). Antisense Oligonucleotide (ASO) technology has developed
636 since then with therapeutic success and some ASO drugs being approved. ASO-mediated modulation
637 of pre-mRNA splicing targets non-productive splicing events. This results in an increase of productive
638 mRNA and upregulation of protein levels (Lim et al., 2020). The technology of dose-dependent
639 upregulation of protein expression is an extremely powerful tool treating dysfunction of proteins due
640 to genetic mutations. Several studies investigated the potential of ASOs in neuromuscular disease
641 (reviewed in the paper (Sardone et al., 2017)). Another study selected four disease-associated genes –
642 one of them *SYNGAP1* - whose transcripts were sensitive to nonsense-mediated mRNA decay (NMD),
643 and systematically identified specific ASOs targeting non-productive alternative splicing. ASO
644 treatment successfully increased protein levels in HEK293 cells as well as in mice (Lim et al., 2020).
645 Importantly, they were able to tightly control upregulation protein levels *in vitro* and *in vivo* in a dose-
646 dependent manner which is key to not risk overexpression. Those examples illustrate that ASO
647 technology is extremely promising. However, there are still limitations to overcome. One is the
648 efficient and precise delivery to the target site, as no other but the target organ should be affected.
649 ASOs have to cross vascular endothelial barrier, eventually the blood-brain barriers without forfeiting
650 stability and specificity. Strategies for ASO delivery are (1) viral delivery, which can cause an immune-
651 response, (2) incorporation into nanoparticles or (3) targeting ligand allowing receptor-mediated
652 endocytosis (Sardone et al., 2017) to only name few. Those different strategies have their advantages
653 and disadvantages and further modifications will optimize the efficacy of ASO technology as a
654 treatment.

655 Conclusion

656 In conclusion, evidence is growing that genetic defects causing neurodevelopmental diseases often
657 target directly or indirectly proteins involved in synaptic structure. These in turn have profound impact
658 on synaptic function. The recent emergence of super-resolution microscopy has provided new insight
659 in synapse nanoscale organization and how it relates to synapse function and plasticity. We posit that
660 many of the gene mutations causing synapse dysfunction may do so by subtle alterations in synapse
661 nanoscale dynamic organization that may not appear obvious at first glance. It is thus necessary to
662 develop the approaches and cellular models that will allow to decipher the impact of synaptic protein
663 dysfunction on synapse organization and link to dysfunction.

664

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671 Competing interests

672 The authors declare no competing interests.

673 Author contributions

674 Both authors contributed equally.

675

676 **References**

677

678 Agrawal, S., 2010. Remembering Paul C. Zamecnik, M.D., "father of antisense" (1912-2009).
679 Oligonucleotides. 20, 47-50. <https://doi.org/10.1089/oli.2009.2001.obz>

680 Aloisi, E., et al., 2017. Altered surface mGluR5 dynamics provoke synaptic NMDAR dysfunction
681 and cognitive defects in Fmr1 knockout mice. Nat Commun. 8, 1103.
682 <https://doi.org/10.1038/s41467-017-01191-2>

683 Antoine, M. W., et al., 2019. Increased Excitation-Inhibition Ratio Stabilizes Synapse and Circuit
684 Excitability in Four Autism Mouse Models. Neuron. 101, 648-661 e4.
685 <https://doi.org/10.1016/j.neuron.2018.12.026>

686 Aoi, T., 2016. 10th anniversary of iPS cells: the challenges that lie ahead. J Biochem. 160, 121-9.
687 <https://doi.org/10.1093/jb/mvw044>

688 Araki, Y., et al., 2015. Rapid dispersion of SynGAP from synaptic spines triggers AMPA receptor
689 insertion and spine enlargement during LTP. Neuron. 85, 173-189.
690 <https://doi.org/10.1016/j.neuron.2014.12.023>

691 Bademosi, A. T., et al., 2017. In vivo single-molecule imaging of syntaxin1A reveals
692 polyphosphoinositide- and activity-dependent trapping in presynaptic nanoclusters. Nat
693 Commun. 8, 13660. <https://doi.org/10.1038/ncomms13660>

694 Baltussen, L. L., et al., 2018. Kinases in synaptic development and neurological diseases. Prog
695 Neuropsychopharmacol Biol Psychiatry. 84, 343-352.
696 <https://doi.org/10.1016/j.pnpbp.2017.12.006>

697 Banke, T. G., Barria, A., 2020. Transient Enhanced GluA2 Expression in Young Hippocampal
698 Neurons of a Fragile X Mouse Model. Front Synaptic Neurosci. 12, 588295.
699 <https://doi.org/10.3389/fnsyn.2020.588295>

700 Bannai, H., et al., 2009. Activity-dependent tuning of inhibitory neurotransmission based on
701 GABAAR diffusion dynamics. Neuron. 62, 670-82. [https://doi.org/S0896-
702 6273\(09\)00347-X](https://doi.org/S0896-6273(09)00347-X) [pii]

703 [10.1016/j.neuron.2009.04.023](https://doi.org/10.1016/j.neuron.2009.04.023)

704 Bariselli, S., et al., 2016. SHANK3 controls maturation of social reward circuits in the VTA. Nat
705 Neurosci. 19, 926-934. <https://doi.org/10.1038/nn.4319>

706 Baude, A., et al., 1995. High-resolution immunogold localization of AMPA type glutamate receptor
707 subunits at synaptic and non-synaptic sites in rat hippocampus. Neuroscience. 69, 1031-
708 55.

709 Baude, A., et al., 1993. The metabotropic glutamate receptors (mGluR1 α) is concentrated at
710 perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction.
711 Neuron. 11, 771-787.

712 Bayer, K. U., Schulman, H., 2019. CaM Kinase: Still Inspiring at 40. Neuron. 103, 380-394.
713 <https://doi.org/10.1016/j.neuron.2019.05.033>

714 Beghin, A., et al., 2017. Localization-based super-resolution imaging meets high-content
715 screening. Nat Methods. 14, 1184-1190. <https://doi.org/10.1038/nmeth.4486>

716 Benarroch, E. E., 2013. HCN channels: function and clinical implications. Neurology. 80, 304-10.
717 <https://doi.org/10.1212/WNL.0b013e31827dec42>

718 Betancur, C., 2011. Etiological heterogeneity in autism spectrum disorders: more than 100 genetic
719 and genomic disorders and still counting. Brain Res. 1380, 42-77.
720 <https://doi.org/10.1016/j.brainres.2010.11.078>

721 Betzig, E., et al., 2006. Imaging intracellular fluorescent proteins at nanometer resolution. Science.
722 313, 1642-5. <https://doi.org/1127344> [pii]

723 [10.1126/science.1127344](https://doi.org/10.1126/science.1127344)

724 Bhakar, A. L., et al., 2012. The pathophysiology of fragile X (and what it teaches us about synapses).
725 Annu Rev Neurosci. 35, 417-43. [https://doi.org/10.1146/annurev-neuro-060909-
726 153138](https://doi.org/10.1146/annurev-neuro-060909-153138)

727 Biederer, T., et al., 2017. Transcellular Nanoalignment of Synaptic Function. *Neuron*. 96, 680-696.
728 <https://doi.org/10.1016/j.neuron.2017.10.006>

729 Booker, S. A., et al., 2019. Altered dendritic spine function and integration in a mouse model of
730 fragile X syndrome. *Nat Commun*. 10, 4813. [https://doi.org/10.1038/s41467-019-](https://doi.org/10.1038/s41467-019-11891-6)
731 [11891-6](https://doi.org/10.1038/s41467-019-11891-6)

732 Borgdorff, A. J., Choquet, D., 2002. Regulation of AMPA receptor lateral movements. *Nature*. 417,
733 649-53.

734 Borodezt, K., D'Mello, S. R., 1998. Decreased expression of the metabotropic glutamate receptor-4
735 gene is associated with neuronal apoptosis. *J Neurosci Res*. 53, 531-41.
736 [https://doi.org/10.1002/\(SICI\)1097-4547\(19980901\)53:5<531::AID-JNR3>3.0.CO;2-A](https://doi.org/10.1002/(SICI)1097-4547(19980901)53:5<531::AID-JNR3>3.0.CO;2-A)

737 Bourgeron, T., 2015. From the genetic architecture to synaptic plasticity in autism spectrum
738 disorder. *Nat Rev Neurosci*. 16, 551-63. <https://doi.org/10.1038/nrn3992>

739 Carta, M., et al., 2013. CaMKII-dependent phosphorylation of GluK5 mediates plasticity of kainate
740 receptors. *EMBO J*. 32, 496-510. <https://doi.org/10.1038/emboj.2012.334>

741 Chamma, I., et al., 2016. Mapping the dynamics and nanoscale organization of synaptic adhesion
742 proteins using monomeric streptavidin. *Nature Communications*. 7.
743 <https://doi.org/10.1038/ncomms10773>

744 Chamma, I., et al., 2020. Biophysical mechanisms underlying the membrane trafficking of synaptic
745 adhesion molecules. *Neuropharmacology*. 169, 107555.
746 <https://doi.org/10.1016/j.neuropharm.2019.02.037>

747 Chen, H., et al., 2018. Subsynaptic spatial organization as a regulator of synaptic strength and
748 plasticity. *Curr Opin Neurobiol*. 51, 147-153.
749 <https://doi.org/10.1016/j.conb.2018.05.004>

750 Chen, H. I., et al., 2019. Applications of Human Brain Organoids to Clinical Problems. *Dev Dyn*. 248,
751 53-64. <https://doi.org/10.1002/dvdy.24662>

752 Chen, H. J., et al., 1998. A synaptic Ras-GTPase activating protein (p135 SynGAP) inhibited by CaM
753 kinase II. *Neuron*. 20, 895-904. [https://doi.org/10.1016/s0896-6273\(00\)80471-7](https://doi.org/10.1016/s0896-6273(00)80471-7)

754 Choquet, D., Hosy, E., 2020. AMPA receptor nanoscale dynamic organization and synaptic
755 plasticities. *Curr Opin Neurobiol*. 63, 137-145.
756 <https://doi.org/10.1016/j.conb.2020.04.003>

757 Choquet, D., Triller, A., 2013. The dynamic synapse. *Neuron*. 80, 691-703.
758 <https://doi.org/10.1016/j.neuron.2013.10.013>

759 Clement, J. P., et al., 2012. Pathogenic SYNGAP1 mutations impair cognitive development by
760 disrupting maturation of dendritic spine synapses. *Cell*. 151, 709-723.
761 <https://doi.org/10.1016/j.cell.2012.08.045>

762 Corti, C., et al., 2002. Distribution and synaptic localisation of the metabotropic glutamate receptor
763 4 (mGluR4) in the rodent CNS. *Neuroscience*. 110, 403-20.
764 [https://doi.org/10.1016/s0306-4522\(01\)00591-7](https://doi.org/10.1016/s0306-4522(01)00591-7)

765 Crosby, K. C., et al., 2019. Nanoscale Subsynaptic Domains Underlie the Organization of the
766 Inhibitory Synapse. *Cell Rep*. 26, 3284-3297 e3.
767 <https://doi.org/10.1016/j.celrep.2019.02.070>

768 Dani, A., et al., 2010. Superresolution imaging of chemical synapses in the brain. *Neuron*. 68, 843-
769 56. <https://doi.org/10.1016/j.neuron.2010.11.021>

770 Dupuis, J. P., et al., 2014. Surface dynamics of GluN2B-NMDA receptors controls plasticity of
771 maturing glutamate synapses. *EMBO J*. 33, 842-61.
772 <https://doi.org/10.1002/emboj.201386356>

773 Ferraguti, F., Shigemoto, R., 2006. Metabotropic glutamate receptors. *Cell Tissue Res*. 326, 483-
774 504. <https://doi.org/10.1007/s00441-006-0266-5>

775 Ferreira, L. M., Mostajo-Radji, M. A., 2013. How induced pluripotent stem cells are redefining
776 personalized medicine. *Gene*. 520, 1-6. <https://doi.org/10.1016/j.gene.2013.02.037>

777 Fukata, Y., et al., 2021. LGI1-ADAM22-MAGUK configures transsynaptic nanoalignment for
778 synaptic transmission and epilepsy prevention. *Proc Natl Acad Sci U S A*. 118.
779 <https://doi.org/10.1073/pnas.2022580118>

780 Fukata, Y., et al., 2013. Local palmitoylation cycles define activity-regulated postsynaptic
781 subdomains. *J Cell Biol*. 202, 145-61. <https://doi.org/10.1083/jcb.201302071>

782 Fukata, Y., et al., 2017. The LGI1-ADAM22 protein complex in synaptic transmission and synaptic
783 disorders. *Neurosci Res.* 116, 39-45. <https://doi.org/10.1016/j.neures.2016.09.011>

784 Gamache, T. R., et al., 2020. Twenty Years of SynGAP Research: From Synapses to Cognition. *J*
785 *Neurosci.* 40, 1596-1605. <https://doi.org/10.1523/JNEUROSCI.0420-19.2020>

786 Giannone, G., et al., 2010. Dynamic superresolution imaging of endogenous proteins on living cells
787 at ultra-high density. *Biophys J.* 99, 1303-10. [https://doi.org/S0006-3495\(10\)00713-7](https://doi.org/S0006-3495(10)00713-7)
788 [pii]
10.1016/j.bpj.2010.06.005

789 Goncalves, J., et al., 2020. Nanoscale co-organization and coactivation of AMPAR, NMDAR, and
790 mGluR at excitatory synapses. *Proc Natl Acad Sci U S A.* 117, 14503-14511.
791 <https://doi.org/10.1073/pnas.1922563117>

792 Good, M. C., et al., 2011. Scaffold proteins: hubs for controlling the flow of cellular information.
793 *Science.* 332, 680-6. <https://doi.org/10.1126/science.1198701>

794 Gou, G., et al., 2020. SynGAP splice variants display heterogeneous spatio-temporal expression and
795 subcellular distribution in the developing mammalian brain. *J Neurochem.* 154, 618-634.
796 <https://doi.org/10.1111/jnc.14988>

797 Gouder, L., et al., 2019. Altered spinogenesis in iPSC-derived cortical neurons from patients with
798 autism carrying de novo SHANK3 mutations. *Sci Rep.* 9, 94.
799 <https://doi.org/10.1038/s41598-018-36993-x>

800 Groc, L., Choquet, D., 2020. Linking glutamate receptor movements and synapse function. *Science.*
801 368. <https://doi.org/10.1126/science.aay4631>

802 Groc, L., et al., 2008. The stress hormone corticosterone conditions AMPAR surface trafficking and
803 synaptic potentiation. *Nat Neurosci.* 11, 868-70.

804 Groc, L., et al., 2007. NMDA receptor surface trafficking and synaptic subunit composition are
805 developmentally regulated by the extracellular matrix protein Reelin. *J Neurosci.* 27,
806 10165-75.

807 Gustafsson, M. G., 2000. Surpassing the lateral resolution limit by a factor of two using structured
808 illumination microscopy. *J Microsc.* 198, 82-7.

809 Haas, K. T., et al., 2018. Pre-post synaptic alignment through neuroligin-1 tunes synaptic
810 transmission efficiency. *Elife.* 7. <https://doi.org/10.7554/eLife.31755>

811 Hafner, A. S., et al., 2015. Lengthening of the Stargazin Cytoplasmic Tail Increases Synaptic
812 Transmission by Promoting Interaction to Deeper Domains of PSD-95. *Neuron.* 86, 475-
813 89. <https://doi.org/10.1016/j.neuron.2015.03.013>

814 Hamdan, F. F., et al., 2009. Mutations in SYNGAP1 in autosomal nonsyndromic mental retardation.
815 *N Engl J Med.* 360, 599-605. <https://doi.org/10.1056/NEJMoa0805392>

816 Heilemann, M., et al., 2008. Subdiffraction-resolution fluorescence imaging with conventional
817 fluorescent probes. *Angew Chem Int Ed Engl.* 47, 6172-6.
818 <https://doi.org/10.1002/anie.200802376>

819 Heine, M., et al., 2008. Activity-independent and subunit-specific recruitment of functional AMPA
820 receptors at neuroligin/neuroligin contacts. *Proc Natl Acad Sci U S A.* 105, 20947-52.
821 <https://doi.org/10.1073/pnas.0804007106>

822 Hell, S. W., Wichmann, J., 1994. Breaking the diffraction resolution limit by stimulated emission:
823 stimulated-emission-depletion fluorescence microscopy. *Opt Lett.* 19, 780-2.
824 <https://doi.org/10.1364/ol.19.000780> [pii]

825 Hess, S. T., et al., 2006. Ultra-high resolution imaging by fluorescence photoactivation localization
826 microscopy. *Biophys J.* 91, 4258-72. [https://doi.org/S0006-3495\(06\)72140-3](https://doi.org/S0006-3495(06)72140-3) [pii]
827 10.1529/biophysj.106.091116

828 Hettige, N. C., Ernst, C., 2019. FOXG1 Dose in Brain Development. *Front Pediatr.* 7, 482.
829 <https://doi.org/10.3389/fped.2019.00482>

830 Holler, S., et al., 2021. Structure and function of a neocortical synapse. *Nature.* 591, 111-116.
831 <https://doi.org/10.1038/s41586-020-03134-2>

832 Hruska, M., et al., 2018. Synaptic nanomodules underlie the organization and plasticity of spine
833 synapses. *Nat Neurosci.* 21, 671-682. <https://doi.org/10.1038/s41593-018-0138-9>
834

835 Huang, Y., Thathiah, A., 2015. Regulation of neuronal communication by G protein-coupled
836 receptors. *FEBS Lett.* 589, 1607-19. <https://doi.org/10.1016/j.febslet.2015.05.007>

837 Huebschman, J. L., et al., 2020. The Fragile X Mental Retardation Protein Regulates Striatal Medium
838 Spiny Neuron Synapse Density and Dendritic Spine Morphology. *Front Mol Neurosci.* 13,
839 161. <https://doi.org/10.3389/fnmol.2020.00161>

840 Iasevoli, F., et al., 2013. Scaffolding proteins of the post-synaptic density contribute to synaptic
841 plasticity by regulating receptor localization and distribution: relevance for
842 neuropsychiatric diseases. *Neurochem Res.* 38, 1-22. <https://doi.org/10.1007/s11064-012-0886-y>

844 Indriati, D. W., et al., 2013. Quantitative localization of Cav2.1 (P/Q-type) voltage-dependent
845 calcium channels in Purkinje cells: somatodendritic gradient and distinct somatic
846 coclustering with calcium-activated potassium channels. *J Neurosci.* 33, 3668-78.
847 <https://doi.org/10.1523/JNEUROSCI.2921-12.2013>

848 Jacob, T. C., et al., 2005. Gephyrin regulates the cell surface dynamics of synaptic GABAA receptors.
849 *J Neurosci.* 25, 10469-78. <https://doi.org/25/45/10469> [pii]
10.1523/JNEUROSCI.2267-05.2005

850 10.1523/JNEUROSCI.2267-05.2005

851 Jamain, S., et al., 2003. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4
852 are associated with autism. *Nat Genet.* 34, 27-9. <https://doi.org/10.1038/ng1136>

853 Jezequel, J., et al., 2017. Dynamic disorganization of synaptic NMDA receptors triggered by
854 autoantibodies from psychotic patients. *Nat Commun.* 8, 1791.
855 <https://doi.org/10.1038/s41467-017-01700-3>

856 Jezequel, J., et al., 2018. Pathogenicity of Antibodies against NMDA Receptor: Molecular Insights
857 into Autoimmune Psychosis. *Trends in Neurosciences.* 41, 502-511.
858 <https://doi.org/10.1016/j.tins.2018.05.002>

859 Kasula, R., et al., 2016. The Munc18-1 domain 3a hinge-loop controls syntaxin-1A nanodomain
860 assembly and engagement with the SNARE complex during secretory vesicle priming. *J*
861 *Cell Biol.* 214, 847-58. <https://doi.org/10.1083/jcb.201508118>

862 Kellermayer, B., et al., 2018. Differential Nanoscale Topography and Functional Role of GluN2-
863 NMDA Receptor Subtypes at Glutamatergic Synapses. *Neuron.* 100, 106-+.
864 <https://doi.org/10.1016/j.neuron.2018.09.012>

865 Kervestin, S., Jacobson, A., 2012. NMD: a multifaceted response to premature translational
866 termination. *Nat Rev Mol Cell Biol.* 13, 700-12. <https://doi.org/10.1038/nrm3454>

867 Kim, E., Sheng, M., 2004. PDZ domain proteins of synapses. *Nat Rev Neurosci.* 5, 771-81.
868 <https://doi.org/10.1038/nrn1517>

869 Kim, J. H., et al., 1998. SynGAP: a synaptic RasGAP that associates with the PSD-95/SAP90 protein
870 family. *Neuron.* 20, 683-91. [https://doi.org/S0896-6273\(00\)81008-9](https://doi.org/S0896-6273(00)81008-9) [pii]

871 Krueger, D. D., Bear, M. F., 2011. Toward fulfilling the promise of molecular medicine in fragile X
872 syndrome. *Annu Rev Med.* 62, 411-29. <https://doi.org/10.1146/annurev-med-061109-134644>

873 134644

874 Kunde, S. A., et al., 2013. Characterisation of de novo MAPK10/JNK3 truncation mutations
875 associated with cognitive disorders in two unrelated patients. *Hum Genet.* 132, 461-71.
876 <https://doi.org/10.1007/s00439-012-1260-5>

877 Lancaster, M. A., Knoblich, J. A., 2014. Generation of cerebral organoids from human pluripotent
878 stem cells. *Nat Protoc.* 9, 2329-40. <https://doi.org/10.1038/nprot.2014.158>

879 Letellier, M., et al., 2018. A unique intracellular tyrosine in neuroligin-1 regulates AMPA receptor
880 recruitment during synapse differentiation and potentiation. *Nat Commun.* 9, 3979.
881 <https://doi.org/10.1038/s41467-018-06220-2>

882 Li, H. L., et al., 2016. Efficient genomic correction methods in human iPS cells using CRISPR-Cas9
883 system. *Methods.* 101, 27-35. <https://doi.org/10.1016/j.ymeth.2015.10.015>

884 Li, J., et al., 2017. Spatiotemporal profile of postsynaptic interactomes integrates components of
885 complex brain disorders. *Nat Neurosci.* 20, 1150-1161. <https://doi.org/10.1038/nn.4594>

886 Li, S., et al., 2021. Asynchronous release sites align with NMDA receptors in mouse hippocampal
887 synapses. *Nat Commun.* 12, 677. <https://doi.org/10.1038/s41467-021-21004-x>

888 Lim, K. H., et al., 2020. Antisense oligonucleotide modulation of non-productive alternative
889 splicing upregulates gene expression. *Nat Commun.* 11, 3501.
890 <https://doi.org/10.1038/s41467-020-17093-9>
891 Llamosas, N., et al., 2020. SYNGAP1 Controls the Maturation of Dendrites, Synaptic Function, and
892 Network Activity in Developing Human Neurons. *J Neurosci.* 40, 7980-7994.
893 <https://doi.org/10.1523/JNEUROSCI.1367-20.2020>
894 Lovero, K. L., et al., 2015. The LGI1-ADAM22 protein complex directs synapse maturation through
895 regulation of PSD-95 function. *Proc Natl Acad Sci U S A.* 112, E4129-37.
896 <https://doi.org/10.1073/pnas.1511910112>
897 Lujan, R., et al., 1996. Perisynaptic location of metabotropic glutamate receptors mGluR1 and
898 mGluR5 on dendrites and dendritic spines in the rat hippocampus. *Eur J Neurosci.* 8, 1488-
899 500. <https://doi.org/10.1111/j.1460-9568.1996.tb01611.x>
900 MacGillavry, H. D., Hoogenraad, C. C., 2018. Membrane trafficking and cytoskeletal dynamics in
901 neuronal function. *Mol Cell Neurosci.* 91, 1-2.
902 <https://doi.org/10.1016/j.mcn.2018.08.003>
903 MacGillavry, H. D., et al., 2013. Nanoscale scaffolding domains within the postsynaptic density
904 concentrate synaptic AMPA receptors. *Neuron.* 78, 615-22.
905 <https://doi.org/10.1016/j.neuron.2013.03.009>
906 Martin, J. P., Bell, J., 1943. A Pedigree of Mental Defect Showing Sex-Linkage. *J Neurol Psychiatry.*
907 6, 154-7. <https://doi.org/10.1136/jnnp.6.3-4.154>
908 Masugi-Tokita, M., Shigemoto, R., 2007. High-resolution quantitative visualization of glutamate
909 and GABA receptors at central synapses. *Curr Opin Neurobiol.* 17, 387-393.
910 Maynard, S. A., Triller, A., 2019. Inhibitory Receptor Diffusion Dynamics. *Front Mol Neurosci.* 12,
911 313. <https://doi.org/10.3389/fnmol.2019.00313>
912 McMahon, A. C., et al., 2012. SynGAP isoforms exert opposing effects on synaptic strength. *Nat*
913 *Commun.* 3, 900. <https://doi.org/10.1038/ncomms1900>
914 Meier, J., et al., 2001. Fast and reversible trapping of surface glycine receptors by gephyrin. *Nat*
915 *Neurosci.* 4, 253-60.
916 Miller, J. N., Pearce, D. A., 2014. Nonsense-mediated decay in genetic disease: friend or foe? *Mutat*
917 *Res Rev Mutat Res.* 762, 52-64. <https://doi.org/10.1016/j.mrrev.2014.05.001>
918 Monteiro, P., Feng, G., 2017. SHANK proteins: roles at the synapse and in autism spectrum
919 disorder. *Nat Rev Neurosci.* 18, 147-157. <https://doi.org/10.1038/nrn.2016.183>
920 Moretto, E., et al., 2019. TSPAN5 Enriched Microdomains Provide a Platform for Dendritic Spine
921 Maturation through Neuroligin-1 Clustering. *Cell Rep.* 29, 1130-1146 e8.
922 <https://doi.org/10.1016/j.celrep.2019.09.051>
923 Mossa, A., et al., 2021. Developmental impaired Akt signaling in the Shank1 and Shank3 double
924 knock-out mice. *Mol Psychiatry.* <https://doi.org/10.1038/s41380-020-00979-x>
925 Mullins, C., et al., 2016. Unifying Views of Autism Spectrum Disorders: A Consideration of
926 Autoregulatory Feedback Loops. *Neuron.* 89, 1131-1156.
927 <https://doi.org/10.1016/j.neuron.2016.02.017>
928 Na, E. S., et al., 2013. The impact of MeCP2 loss- or gain-of-function on synaptic plasticity.
929 *Neuropsychopharmacology.* 38, 212-9. <https://doi.org/10.1038/npp.2012.116>
930 Nair, D., et al., 2013. Super-Resolution Imaging Reveals That AMPA Receptors Inside Synapses Are
931 Dynamically Organized in Nanodomains Regulated by PSD95. *Journal of Neuroscience.* 33,
932 13204-13224. <https://doi.org/10.1523/jneurosci.2381-12.2013>
933 Naisbitt, S., et al., 1999. Shank, a novel family of postsynaptic density proteins that binds to the
934 NMDA receptor/PSD-95/GKAP complex and cortactin. *Neuron.* 23, 569-82.
935 Nakanishi, M., et al., 2017. Functional significance of rare neuroligin 1 variants found in autism.
936 *PLoS Genet.* 13, e1006940. <https://doi.org/10.1371/journal.pgen.1006940>
937 Nguyen, T. A., et al., 2020. Neuroligins and Neurodevelopmental Disorders: X-Linked Genetics.
938 *Front Synaptic Neurosci.* 12, 33. <https://doi.org/10.3389/fnsyn.2020.00033>
939 Nicoll, R. A., 2017. A Brief History of Long-Term Potentiation. *Neuron.* 93, 281-290.
940 <https://doi.org/10.1016/j.neuron.2016.12.015>
941 Nusser, Z., et al., 1994. Subsynaptic segregation of metabotropic and ionotropic glutamate
942 receptors as revealed by immunogold localization. *Neuroscience.* 61, 421-427.

943 Opazo, P., et al., 2018. CaMKII Metaplasticity Drives A beta Oligomer-Mediated Synaptotoxicity.
944 Cell Reports. 23, 3137-3145. <https://doi.org/10.1016/j.celrep.2018.05.036>

945 Opazo, P., et al., 2010. CaMKII triggers the diffusional trapping of surface AMPARs through
946 phosphorylation of stargazin. Neuron. 67, 239-52.
947 <https://doi.org/10.1016/j.neuron.2010.06.007>

948 Opazo, P., et al., 2012. Regulation of AMPA receptor surface diffusion by PSD-95 slots. Curr Opin
949 Neurobiol. 22, 453-60. <https://doi.org/10.1016/j.conb.2011.10.010>

950 Padmanabhan, P., et al., 2020. Need for speed: Super-resolving the dynamic nanoclustering of
951 syntaxin-1 at exocytic fusion sites. Neuropharmacology. 169, 107554.
952 <https://doi.org/10.1016/j.neuropharm.2019.02.036>

953 Pardo, M., et al., 2017. Cotinine administration improves impaired cognition in the mouse model
954 of Fragile X syndrome. Eur J Neurosci. 45, 490-498. <https://doi.org/10.1111/ejn.13446>

955 Parenti, I., et al., 2020. Neurodevelopmental Disorders: From Genetics to Functional Pathways.
956 Trends Neurosci. 43, 608-621. <https://doi.org/10.1016/j.tins.2020.05.004>

957 Patzke, C., Sudhof, T. C., 2016. The conditional KO approach: Cre/Lox technology in human
958 neurons. Rare Dis. 4, e1131884. <https://doi.org/10.1080/21675511.2015.1131884>

959 Pekhletski, R., et al., 1996. Impaired cerebellar synaptic plasticity and motor performance in mice
960 lacking the mGluR4 subtype of metabotropic glutamate receptor. J Neurosci. 16, 6364-73.

961 Petit-Pedrol, M., Groc, L., 2021. Regulation of membrane NMDA receptors by dynamics and protein
962 interactions. J Cell Biol. 220. <https://doi.org/10.1083/jcb.202006101>

963 Petrini, E. M., Barberis, A., 2014. Diffusion dynamics of synaptic molecules during inhibitory
964 postsynaptic plasticity. Front Cell Neurosci. 8, 300.
965 <https://doi.org/10.3389/fncel.2014.00300>

966 Petrini, E. M., et al., 2014. Synaptic recruitment of gephyrin regulates surface GABAA receptor
967 dynamics for the expression of inhibitory LTP. Nat Commun. 5, 3921.
968 <https://doi.org/10.1038/ncomms4921>

969 Ramon y Cajal, S., 1899. Textura del Sistema Nervioso del Hombre y de los Vertebrados: Estudios
970 Sobre el Plan Estructural y Composición Histológica de los Centros Nerviosos Adicionados
971 de Consideraciones Fisiológicas Fundadas en los Nuevos Descubrimientos. Moya, Madrid.

972 Ribault, C., et al., 2011. Syntaxin1A lateral diffusion reveals transient and local SNARE
973 interactions. J Neurosci. 31, 17590-602. <https://doi.org/10.1523/JNEUROSCI.4065-11.2011>

974

975 Rothwell, P. E., et al., 2014. Autism-associated neuroligin-3 mutations commonly impair striatal
976 circuits to boost repetitive behaviors. Cell. 158, 198-212.
977 <https://doi.org/10.1016/j.cell.2014.04.045>

978 Rumbaugh, G., et al., 2006. SynGAP regulates synaptic strength and mitogen-activated protein
979 kinases in cultured neurons. Proc Natl Acad Sci U S A. 103, 4344-51.
980 <https://doi.org/10.1073/pnas.0600084103>

981 Rust, M. J., et al., 2006. Sub-diffraction-limit imaging by stochastic optical reconstruction
982 microscopy (STORM). Nat Methods. 3, 793-5. <https://doi.org/nmeth929> [pii]

983 10.1038/nmeth929

984 Sajman, J., et al., 2017. The L-type Voltage-Gated Calcium Channel co-localizes with Syntaxin 1A in
985 nano-clusters at the plasma membrane. Sci Rep. 7, 11350.
986 <https://doi.org/10.1038/s41598-017-10588-4>

987 Sardone, V., et al., 2017. Antisense Oligonucleotide-Based Therapy for Neuromuscular Disease.
988 Molecules. 22. <https://doi.org/10.3390/molecules22040563>

989 Schneider, R., et al., 2015. Mobility of calcium channels in the presynaptic membrane. Neuron. 86,
990 672-9. <https://doi.org/10.1016/j.neuron.2015.03.050>

991 Schwenk, J., et al., 2012. High-resolution proteomics unravel architecture and molecular diversity
992 of native AMPA receptor complexes. Neuron. 74, 621-33. [https://doi.org/S0896-6273\(12\)00345-5](https://doi.org/S0896-6273(12)00345-5) [pii]

993 10.1016/j.neuron.2012.03.034

994 Selkoe, D. J., 2002. Alzheimer's disease is a synaptic failure. Science. 298, 789-91.
995 <https://doi.org/10.1126/science.1074069>

996

997 298/5594/789 [pii]
998 Shcheglovitov, A., et al., 2013. SHANK3 and IGF1 restore synaptic deficits in neurons from 22q13
999 deletion syndrome patients. *Nature*. 503, 267-71. <https://doi.org/10.1038/nature12618>
1000 Shigemoto, R., et al., 1996. Target-cell-specific concentration of a metabotropic glutamate receptor
1001 in the presynaptic active zone. *Nature*. 381, 523-5. <https://doi.org/10.1038/381523a0>
1002 Siddig, S., et al., 2020. Super-resolution imaging reveals the nanoscale organization of
1003 metabotropic glutamate receptors at presynaptic active zones. *Sci Adv*. 6, eaay7193.
1004 <https://doi.org/10.1126/sciadv.aay7193>
1005 Sieber, J. J., et al., 2006. The SNARE motif is essential for the formation of syntaxin clusters in the
1006 plasma membrane. *Biophys J*. 90, 2843-51.
1007 Specht, C. G., et al., 2013. Quantitative nanoscopy of inhibitory synapses: counting gephyrin
1008 molecules and receptor binding sites. *Neuron*. 79, 308-21.
1009 <https://doi.org/10.1016/j.neuron.2013.05.013>
1010 Stephenson, M. L., Zamecnik, P. C., 1978. Inhibition of Rous sarcoma viral RNA translation by a
1011 specific oligodeoxyribonucleotide. *Proc Natl Acad Sci U S A*. 75, 285-8.
1012 <https://doi.org/10.1073/pnas.75.1.285>
1013 Stoddard-Bennett, T., Reijo Pera, R., 2019. Treatment of Parkinson's Disease through Personalized
1014 Medicine and Induced Pluripotent Stem Cells. *Cells*. 8.
1015 <https://doi.org/10.3390/cells8010026>
1016 Stornetta, R. L., Zhu, J. J., 2011. Ras and Rap signaling in synaptic plasticity and mental disorders.
1017 *Neuroscientist*. 17, 54-78. <https://doi.org/10.1177/1073858410365562>
1018 Sudhof, T. C., 2018. Towards an Understanding of Synapse Formation. *Neuron*. 100, 276-293.
1019 <https://doi.org/10.1016/j.neuron.2018.09.040>
1020 Tang, A. H., et al., 2016. A trans-synaptic nanocolumn aligns neurotransmitter release to receptors.
1021 *Nature*. 536, 210-4. <https://doi.org/10.1038/nature19058>
1022 Tardin, C., et al., 2003. Direct imaging of lateral movements of AMPA receptors inside synapses.
1023 *Embo J*. 22, 4656-65. <https://doi.org/10.1093/emboj/cdg463>
1024 Tatavarthy, V., et al., 2020. Autism-Associated Shank3 Is Essential for Homeostatic Compensation
1025 in Rodent V1. *Neuron*. 106, 769-777 e4. <https://doi.org/10.1016/j.neuron.2020.02.033>
1026 Triller, A., Choquet, D., 2005. Surface trafficking of receptors between synaptic and extrasynaptic
1027 membranes: and yet they do move! *Trends Neurosci*. 28, 133-9.
1028 Triller, A., et al., 1985. Distribution of glycine receptors at central synapses: an immunoelectron
1029 microscopy study. *J Cell Biol*. 101, 683-8.
1030 Turrigiano, G. G., et al., 1998. Activity-dependent scaling of quantal amplitude in neocortical
1031 neurons. *Nature*. 391, 892-6. <https://doi.org/10.1038/36103>
1032 Vazquez, L. E., et al., 2004. SynGAP regulates spine formation. *J Neurosci*. 24, 8862-72.
1033 <https://doi.org/10.1523/JNEUROSCI.3213-04.2004>
1034 Vicidomini, G., et al., 2018. STED super-resolved microscopy. *Nat Methods*. 15, 173-182.
1035 <https://doi.org/10.1038/nmeth.4593>
1036 Volk, L., et al., 2015. Glutamate synapses in human cognitive disorders. *Annu Rev Neurosci*. 38,
1037 127-49. <https://doi.org/10.1146/annurev-neuro-071714-033821>
1038 Walkup, W. G., et al., 2016. A model for regulation by SynGAP-alpha1 of binding of synaptic
1039 proteins to PDZ-domain 'Slots' in the postsynaptic density. *Elife*. 5.
1040 <https://doi.org/10.7554/eLife.16813>
1041 Wen, Z., et al., 2014. Synaptic dysregulation in a human iPS cell model of mental disorders. *Nature*.
1042 515, 414-8. <https://doi.org/10.1038/nature13716>
1043 Wiesner, T., et al., 2020. Activity-Dependent Remodeling of Synaptic Protein Organization
1044 Revealed by High Throughput Analysis of STED Nanoscopy Images. *Front Neural Circuits*.
1045 14, 57. <https://doi.org/10.3389/fncir.2020.00057>
1046 Wijetunge, L. S., et al., 2014. Stimulated emission depletion (STED) microscopy reveals nanoscale
1047 defects in the developmental trajectory of dendritic spine morphogenesis in a mouse
1048 model of fragile X syndrome. *J Neurosci*. 34, 6405-12.
1049 <https://doi.org/10.1523/JNEUROSCI.5302-13.2014>

1050 Wu, Y., Shroff, H., 2018. Faster, sharper, and deeper: structured illumination microscopy for
1051 biological imaging. *Nat Methods*. 15, 1011-1019. [https://doi.org/10.1038/s41592-018-](https://doi.org/10.1038/s41592-018-0211-z)
1052 [0211-z](https://doi.org/10.1038/s41592-018-0211-z)

1053 Xu, Y., et al., 2021. Proteomic insights into synaptic signaling in the brain: the past, present and
1054 future. *Mol Brain*. 14, 37. <https://doi.org/10.1186/s13041-021-00750-5>

1055 Yi, F., et al., 2016. Autism-associated SHANK3 haploinsufficiency causes Ih channelopathy in
1056 human neurons. *Science*. 352, aaf2669. <https://doi.org/10.1126/science.aaf2669>

1057 Zamecnik, P. C., Stephenson, M. L., 1978. Inhibition of Rous sarcoma virus replication and cell
1058 transformation by a specific oligodeoxynucleotide. *Proc Natl Acad Sci U S A*. 75, 280-4.
1059 <https://doi.org/10.1073/pnas.75.1.280>

1060 Zaslavsky, K., et al., 2019. SHANK2 mutations associated with autism spectrum disorder cause
1061 hyperconnectivity of human neurons. *Nat Neurosci*. 22, 556-564.
1062 <https://doi.org/10.1038/s41593-019-0365-8>

1063 Zeng, M., et al., 2019. Phase Separation-Mediated TARP/MAGUK Complex Condensation and
1064 AMPA Receptor Synaptic Transmission. *Neuron*. 104, 529-543 e6.
1065 <https://doi.org/10.1016/j.neuron.2019.08.001>

1066 Zeng, M., et al., 2016. Phase Transition in Postsynaptic Densities Underlies Formation of Synaptic
1067 Complexes and Synaptic Plasticity. *Cell*. 166, 1163-1175 e12.
1068 <https://doi.org/10.1016/j.cell.2016.07.008>

1069 Zhang, H. Y., et al., 2018. Modulation of AMPA receptor surface diffusion restores hippocampal
1070 plasticity and memory in Huntington's disease models. *Nature Communications*. 9.
1071 <https://doi.org/10.1038/s41467-018-06675-3>

1072 Zoghbi, H. Y., Bear, M. F., 2012. Synaptic dysfunction in neurodevelopmental disorders associated
1073 with autism and intellectual disabilities. *Cold Spring Harb Perspect Biol*. 4.
1074 <https://doi.org/10.1101/cshperspect.a009886>

1075

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